

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
30 September 2004 (30.09.2004)

PCT

(10) International Publication Number
WO 2004/083243 A1

(51) International Patent Classification⁷: **C07K 14/575**,
7/06, A61K 38/22, A61P 5/48

(21) International Application Number:
PCT/EP2004/050320

(22) International Filing Date: 17 March 2004 (17.03.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
03100683.6 18 March 2003 (18.03.2003) EP

(71) Applicant (for all designated States except US): **AP-
PLIED RESEARCH SYSTEMS ARS HOLDING N.V.**
[NL/NL]; Pietermaai 15, Curacao (AN).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **FEZOU, Youcef**
[US/CH]; 7 Rue Jean Pelletier, CH-1225 Chêne-Bourg
(CH). **SOTO-JARA, Claudio** [CL/US]; 2403 Pine Drive,
Friendswood, TX 77546 (US).

(74) Agent: **SERONO INTERNATIONAL SA Giulia Tagli-
affico**; Intellectual Property SA, 12, Chemin des Aulx,
CH-1228 Plan-les-Ouates (CH).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), Euro-
pean (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR,
GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: **AMYLIN AGGREGATION INHIBITORS AND USE THEREOF.**

(57) Abstract: Short peptides and derivatives or analogs thereof for the treatment or prevention of IAAP related disorders, in partic-
ular amyloid deposits associated with Type I or Type II diabetes are herein described. These peptides and/or their derivatives have
been designed to inhibit the formation of amylin fibrils which are implicated in the pathogenesis of diabetes Type II as well as to
dissolve the fibrillar deposits already formed

WO 2004/083243 A1

Amylin aggregation inhibitors and use thereof.**Field of the invention**

The invention relates to the field of β -sheet breaking peptides, particularly to their use
5 in the treatment of Type II diabetes.

Background of the invention

Type-II diabetes is a heterogeneous and multi-factorial disease characterized by beta-cell failure, insulin resistance, and the presence of amyloid deposits in the pancreatic islets of about 90% of patients (*Anguinao et al., 2002*). The major component of these
10 deposits is a 37 amino acid peptide called islet amyloid polypeptide (IAPP) or amylin (*Cooper et al., 1987*).

Amylin is a normal secretory product of the pancreatic beta-cells, stored in the same granules as insulin and is co-released with insulin during the process of exocytosis. The
15 normal function of soluble amylin is to control glucose homeostasis, possibly as an insulin counter-regulatory hormone (*Rink et al., 1993*). However, under certain conditions, the increase in amylin secretion leads to formation amyloid fibrils that deposit in the pancreas of Type II diabetes patients. The formation of amyloid deposits is strongly associated with continuous decline of beta-cell function and the progression
20 of the disease (*Höppener et al., 2000*). A strong evidence for the importance of amyloid in the pathogenesis of diabetes Type II comes from genetic studies showing that mutations in amylin gene result in early onset hereditary disease, specially when accompanied with obesity.

25 IAPP fibril formation has also been found in patients with Type I diabetes post-transplantation. Therefore, the amyloidogenic protein IAPP, that has been shown to induce β -islet cell toxicity *in vitro*, may contribute to the loss of β -islet cells (Langhorns) and organ dysfunction by the formation of fibrils in the pancreas of Type I or Type II diabetic patients.

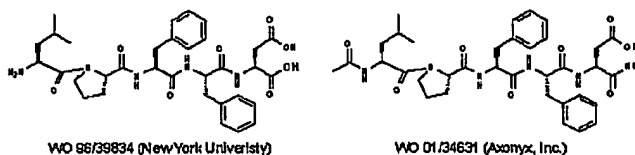
30 Amylin fibril formation involves the conversion of an irregularly structured conformer (random coil, soluble form) into highly stable β -sheet-rich fibrillar aggregates. As in the case of amyloid beta in Alzheimer's disease, the random-coil to β -sheet conversion

is believed to be one of the earliest events in amylin fibril formation. Therefore, diabetes Type II is included in the group of diseases in which protein misfolding and aggregation is a hallmark event in the disease (*Soto et al., 2000; Carrell et al., 1997 and Dobson, 1999*). The central region of amylin sequence, residues 20-29, has been found to be very
 5 important for fibril formation (*Glenner et al., 1988 and Westermark et al., 1990*) and thus might be the sequence where the conversion to β -sheet occurs.

Some fragments of native amylin have been developed as modulators of IAPP aggregation (EP 885904 and US 2002/0119926). In addition, some analogues of amylin
 10 have been developed as amylin agonists for the treatment of hypoglycaemic conditions in which enhanced amylin action is of benefit (EP 1162207 and US 6,610,824).

A human amylin analogue of 37 amino acid, Pramlintide acetate (Symlin®) is being developed by Amylin Pharmaceuticals Inc. as an adjunct with insulin for the potential
 15 prevention of complications of Type I diabetes and as a single agent for Type II diabetes.

One approach to the treatment and prevention of disorders associated with protein misfolding and aggregation has been to develop short peptides having some sequence
 20 homology to the natural protein sequence believed to be involved in amyloid formation, but also having one or more amino acids that disfavour or destabilise the formation of β -pleated sheet conformations. The peptides prevent the aggregation of β -amyloid, and thereby prevent its cytotoxic effects. This approach has been suggested in Alzheimer's disease and in prion-related disorders (WO 96/39834, New York University and WO
 25 01/34631, Axonyx Inc.) leading to the development of the β -sheet breaking peptides shown below, amongst others:



The amino acid proline has been used frequently to destabilize the formation of β -sheet structure, because its physicochemical and structural properties determine that this residue is rarely found inside β -sheet structures. Interestingly, a comparison of the amylin sequence from different species shows that species where a diabetic condition has been reported (human and cats) have a lower number of prolines in the 20-29 region of amylin compared with rodents where amyloid deposition and diabetes has not been shown (Fig. 1) (*Moriarty et al., 1999*). These findings are consistent with the role of amylin aggregation in the pathogenesis of diabetes Type II and further support the concept of β -sheet breaker peptides useful as inhibitors of amyloid formation.

Summary of the invention

It is an object of the invention to provide substances which are suitable for the treatment of and/or prevention of and/or delaying the progression of diabetes, notably Type II diabetes.

It is also an object of the invention to provide substances which are suitable for reducing or inhibiting amylin aggregation.

It is notably an object of the invention to provide β -sheet breaking peptides which are suitable for reducing or inhibiting amyloid formation by amylin.

In a first aspect, the invention provides peptides having an amino acid sequence of Formula I (SEQ ID NO. 1):

$X_1FGAPX_2X_3$ in which

X_1 , is selected from Aspartic acid and a derivative thereof;

X_2 is Leucine or when X_3 is absent, X_2 is selected from Leucine and a derivative thereof;

X_3 can be absent or is selected from Aspartic acid and a derivative thereof;

wherein F represents Phenylalanine, G represents Glycine, A represents Alanine and P represents Proline.

In a second aspect, the present invention provides compounds of Formula I for use as a medicament.

5 In a third aspect, the invention provides a pharmaceutical composition comprising a compound of Formula I, together with a pharmaceutically acceptable excipient or carrier.

In a fourth aspect, the invention provides a use of Formula I for the preparation of a medicament for the treatment and/or prevention of a diabetic condition selected from
10 post-transplantation Type I diabetes and Type II diabetes.

In a fifth aspect, the invention provides a method for treating a disease associated with abnormal folding of the islet amyloid polypeptide.

15 In a sixth aspect, the invention provides a method for treating a patient suffering from diabetes, notably Type II diabetes.

Detailed description of the invention

The following paragraphs provide definitions of various chemical moieties and terms,
20 and are intended to apply uniformly throughout the specification and claims unless an otherwise expressly set out definition provides a different definition.

The term "peptide" is ordinarily applied to a polypeptidic chain containing from 3 to 30 or more contiguous amino acids, usually from 3 to 20 contiguous amino acids. Such
25 peptides can be generated by methods known to those skilled in the art, including partial proteolytic cleavage of a larger protein, chemical synthesis, or genetic engineering.

The expression "derivative or analogue" means any compound the chemical structure of which contains modifications with respect to the parent peptide, but which maintains at
30 least 50%, more preferably at least 75%, most preferably at least 90% of the biological activity of a compound of Formula I.

The term "derivatives" as herein used refers to derivatives which can be prepared from the functional groups present on the lateral chains of the amino acid moieties or on the N-/ or C-terminal groups according to known methods. Such derivatives include for example esters or aliphatic amides of the carboxyl-groups and N-acyl derivatives of free amino groups or O-acyl derivatives of free hydroxyl-groups and are formed with acyl-groups as for example alcanoyl- or aroyl-groups.

The term "salts" herein refers to both salts of carboxyl groups and to acid addition salts of amino groups of the peptides, polypeptides, or analogs thereof, of the present invention. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, arginine or lysine, piperidine, procaine and the like. Acid addition salts include, for example, salts with mineral acids such as, for example, hydrochloric acid or sulfuric acid, and salts with organic acids such as, for example, acetic acid or oxalic acid. Any of such salts should have substantially similar activity to the peptides and polypeptides of the invention or their analogs.

"C₁-C₆-alkyl" refers to monovalent alkyl groups having 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, *tert*-butyl, n-hexyl and the like. By analogy C₁-C₅-alkyl refers to monovalent alkyl groups having 1 to 5 carbon atoms.

"C₁-C₆ Acyl" refers to a group -C(O)R where R includes H and "C₁-C₅-alkyl" groups. This term includes formyl (-C(O)H) and acetyl (-C(O)CH₃).

"Amino" refers to the group -NRR' where each R,R' is independently hydrogen or "C₁-C₆-alkyl".

"Halogen" refers to fluoro, chloro, bromo and iodo atoms.

"Amidated amino acid" refers to an amino acid wherein the hydroxy group from the acid moiety has been replaced by an amino group.

5 "Acylated amino acid" refers to an amino acid wherein the one hydrogen atom on the nitrogen has been replaced by a C₁-C₆ acyl group. Further, an "acetylated amino acid" refers to an amino acid wherein the one hydrogen atom on the nitrogen has been replaced by an acetyl group.

10 "Substituted" refers to groups substituted with from 1 to 5 substituents selected from the group comprising "C₁-C₆-alkyl", "amino", "halogen", trihalomethyl, cyano, hydroxy, mercapto, nitro, and the like.

The polypeptides and the peptides of the present invention can be in other alternative forms which can be preferred according to the desired method of use and/or production,
15 for example as active fractions, precursors, salts, derivatives, conjugates or complexes.

Compounds of the invention are suitable for the treatment and/or prevention of a diabetic condition, including a disease associated with abnormal folding of the islet amyloid polypeptide, Type I or Type II diabetes and post-transplantation Type I or Type
20 II diabetes.

The compounds of the invention may be prepared by any well-known procedure in the art, including chemical synthesis technologies.

25 Examples of chemical synthesis technologies are solid phase synthesis and liquid phase synthesis. As a solid phase synthesis, for example, the amino acid corresponding to the C-terminus of the peptide to be synthesized is bound to a support which is insoluble in organic solvents, and by alternate repetition of reactions, one wherein amino acids with their amino groups and side chain functional groups protected with appropriate
30 protective groups are condensed one by one in order from the C-terminus to the N-terminus, and one where the amino acids bound to the resin or the protective group of the amino groups of the peptides are released, the peptide chain is thus extended in this

manner. Solid phase synthesis methods are largely classified by the tBoc method and the Fmoc method, depending on the type of protective group used. Typically used protective groups include tBoc (t-butoxycarbonyl), Cl-Z (2-chlorobenzyloxycarbonyl), Br-Z (2-bromobenzyloxycarbonyl), Bzl (benzyl), Fmoc (9-fluorenylmethoxycarbonyl), Mbh (4,4'-dimethoxydibenzhydryl), Mtr (4-methoxy-2,3,6-trimethylbenzenesulphonyl), Trt (trityl), Tos (tosyl), Z (benzyloxycarbonyl) and Cl₂-Bzl (2,6-dichlorobenzyl) for the amino groups; NO₂ (nitro) and Pmc (2,2,5,7,8-pentamethylchromane-6-sulphonyl) for the guanidino groups; and tBu (t-butyl) for the hydroxyl groups). After synthesis of the desired peptide, it is subjected to the de-protection reaction and cut out from the solid support. Such peptide cutting reaction may be carried with hydrogen fluoride or tri-fluoromethane sulfonic acid for the Boc method, and with TFA for the Fmoc method.

The present invention provides compounds capable of controlling IAPP aggregation and fibril formation

The activity of the compound of the invention in inhibiting the amylin fibrils can be detected using, for example, an *in vitro* assay, such as that described by *Glunk et al., 1999 and Fozouli et al., 1999*, which measures the ability of compounds of the invention to prevent the aggregation of the islet amyloid polypeptide (IAPP). Results are reported in the Examples.

Amylin fibrils are cytotoxic, inducing cell death by apoptosis (*Lorenzo et al., 1994*). Compounds of the invention can be tested for their ability to prevent cytotoxicity of amylin fibrils. Results are reported in the Examples.

In one embodiment, the invention provides compounds according to Formula I, wherein X₁ is selected from Aspartic acid and acetylated Aspartic acid.

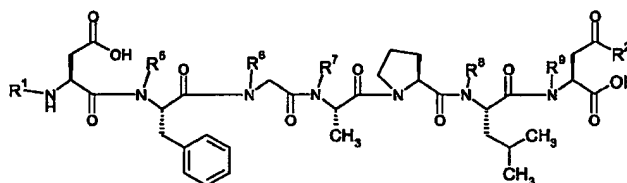
In another embodiment, the invention provides compounds according to Formula I, wherein X₂ is selected from Leucine and amidated Leucine.

In another embodiment, the invention provides compounds according to Formula I, wherein X_3 is absent.

In another embodiment, the invention provides compounds according to Formula I, wherein X_1 is Aspartic acid and X_2 is Leucine.

In another embodiment, the invention provides compounds according to Formula I, wherein X_1 is acetylated Aspartic acid and X_2 is amidated Leucine.

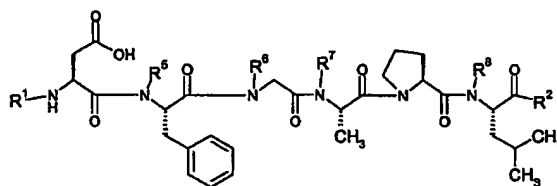
In another embodiment, peptides of the invention are of following Formula II:



Formula II

wherein R^1 is selected from H, optionally substituted C_2-C_6 acyl and optionally substituted C_1-C_6 alkyl, preferably H and acetyl; R^2 is selected from OH and NR^3R^4 , wherein R^3 and R^4 are independently selected from H and optionally substituted C_1-C_6 alkyl, preferably R^2 is selected from OH and NH_2 ; R^5 , R^6 , R^7 , R^8 and R^9 are independently selected from H and C_1-C_6 alkyl.

In another embodiment, peptides of the invention are of following Formula III:



Formula III

wherein R¹ is selected from H, optionally substituted C₂-C₆ acyl and optionally substituted C₁-C₆ alkyl, preferably H and acetyl and R² is selected from OH and NR³R⁴, wherein R³ and R⁴ are independently selected from H and optionally substituted C₁-C₆ alkyl, preferably R² is selected from OH and NH₂; R⁵, R⁶, R⁷ and R⁸ are independently selected from H and C₁-C₆ alkyl.

In another embodiment, the invention provides compounds according to Formulae II or III, wherein R⁵, R⁶, R⁷ and R⁸ are H.

In another embodiment, the invention provides compounds according to Formulae II or III, wherein R¹ is H and R² is OH.

In another embodiment, the invention provides compounds according to Formulae II or III, wherein R¹ is acetyl.

In another embodiment, the invention provides compounds according to Formulae II or III, wherein R² is NH₂.

In another embodiment, the invention provides compounds according to Formula III wherein R¹ is acetyl and R² is NH₂.

In another embodiment of the invention, peptides of Formula I are selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 3 and SEQ ID NO. 4.

In another embodiment of the invention, peptides of Formula I can be used for the preparation of a medicament for the treatment or prevention of IAPP related disorders such as diabetes Type I or Type II, e.g. to prevent or delay the aggregation of amylin associated with the onset and/or progression of Type II diabetes or for the treatment of islet cells, e.g. cultured pancreatic islet cells *in vitro* prior to their transplantation, for the treatment of Type I diabetes patients, e.g., post-transplantation, to prevent or inhibit fibril formation in the transplanted cells.

Still another embodiment of the present invention, is a method for treating or preventing an IAPP related amyloidosis, such as a diabetic disorder, preferably Type II diabetes or post-transplantation Type I diabetes.

5

A further embodiment of the invention is a method for treating or preventing IAPP disorders wherein the method comprises administering an effective dose of the above-mentioned peptides and derivatives thereof to a subject in the need thereof, wherein the subject can be human or animal, preferably human.

10

A further embodiment of the invention comprises the administration of at least a compound of the invention in a regimen coordinated with insulin or with glucose sensitizers, e.g. in a treatment for diabetes for simultaneous, sequential or separate use.

15

Pharmaceutical compositions comprising at least one peptide of the invention include all compositions wherein the peptide(s) are contained in an amount effective to achieve the intended purpose. In addition, the pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Suitable pharmaceutically acceptable vehicles are well known in the art and are described for example in *Gennaro et al, 2000*, a standard reference text in this field. Pharmaceutically acceptable vehicles can be routinely selected in accordance with the mode of administration, solubility and stability of the peptides. For example, formulations for intravenous administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives. The use of biomaterials and other polymers for drug delivery, as well the different techniques and models to validate a specific mode of administration, are disclosed in literature (*Luo et al., 2001 and Cleland et al., 2001*).

20

25

30

The above-mentioned peptides and derivatives of the present invention may be administered by any means that achieve the intended purpose. For example, administration may be achieved by a number of different routes including, but not

limited to subcutaneous, intravenous, intradermal, intramuscular, intracritoneal, intracerebral, intrathecal, intranasal, oral, rectal, transdermal, intranasal or buccal. Preferably the compounds of the invention are administered by subcutaneous, intramuscular or intravenous injection or infusion.

5

Parenteral administration can be by bolus injection or by gradual perfusion over time. A typical regimen for preventing, suppressing, or treating amylin misfolding related disorders, comprises either (1) administration of an effective amount in one or two doses of a high concentration of inhibitory peptides in the range of 0.5 to 10 mg of peptide, more preferably 0.5 to 5 mg of peptide, or (2) administration of an effective amount of the peptide in multiple doses of lower concentrations of inhibitor peptides in the range of 10-1000 μ g, more preferably 50-500 μ g over a period of time up to and including several months to several years. It is understood that the dosage administered will be dependent upon the age, sex, health, and weight of the recipient, concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The total dose required for each treatment may be administered by multiple doses or in a single dose.

15

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain auxiliary agents or excipients which are known in the art. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspension of the active compound as appropriate oily injections suspensions may be administered.

20

Depending on the intended route of delivery, the compounds may be formulated as injectable or oral compositions. The compositions for oral administration can take the form of bulk liquid solutions or suspensions, or bulk powders. More commonly, however, the compositions are presented in unit dosage forms to facilitate accurate dosing. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic

25

30

effect, in association with a suitable pharmaceutical excipient. Typical unit dosage forms include pre-filled, pre-measured ampoules or syringes of the liquid compositions or pills, tablets, capsules or the like in the case of solid compositions. In such compositions, the compound of the invention is usually a minor component (from about 5 0.1 to about 50% by weight or preferably from about 1 to about 40% by weight) with the remainder being various vehicles or carriers and processing aids helpful for forming the desired dosing form.

Liquid forms suitable for oral administration may include a suitable aqueous or 10 non-aqueous vehicle with buffers, suspending and dispensing agents, colorants, flavours and the like. Solid forms may include, for example, any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatine; an excipient such as starch or lactose; a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate; a 15 glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavouring agent such as peppermint, methyl salicylate, or orange flavouring.

Injectable compositions are typically based upon injectable sterile saline or phosphate- 20 buffered saline or other injectable carriers known in the art.

The above-described components for orally administered or injectable compositions are merely representative. Further materials as well as processing techniques and the like are known to the skilled practitioner (*Gennaro et al., 2000*).

25 The compounds of this invention can also be administered in sustained release forms or from sustained release drug delivery systems. A description of representative sustained release materials is also known to the skilled practitioner (*Karsa et al., 1993 and Yacobi et al., 1998*).

30 By "effective amount", is meant an amount sufficient to achieve a concentration of peptide(s) which is capable of slowing down or inhibiting the formation of amylin deposits, or of dissolving pre-formed deposits. Such concentrations can be routinely

determined by those of skilled in the art. The amount of the compound actually administered will typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like. It will also be appreciated by those of skilled in the art that the dosage may be dependent on the stability of the administered peptide. A less stable peptide may require administration in multiple doses.

10 The expression "Pharmaceutically acceptable" is meant to encompass any carrier, which does not interfere with the effectiveness of the biological activity of the active ingredient and that is not toxic to the host to which is administered. For example, for parenteral administration, the above active ingredients may be formulated in unit dosage form for injection in vehicles such as saline, dextrose solution, serum albumin and
15 Ringer's solution.

Besides the pharmaceutically acceptable carrier, the compositions of the invention can also comprise minor amounts of additives, such as stabilizers, excipients, buffers and preservatives.

20 The present invention has been described with reference to the specific embodiments, but the content of the description comprises all modifications and substitutions, which can be brought by a person skilled in the art without extending beyond the meaning and purpose of the claims.

25 The invention will now be described by means of the following Examples, which should not be construed as in any way limiting the present invention. The Examples will refer to the Figures specified here below.

30

Brief description of the drawings:

Fig. 1 compares sequences of human, cat, mouse, rat and hamster amylin. The amyloidogenic domain spanning residues 20-29 is shown by arrows and the sequence used as a template to generate β -sheet breaker peptides is highlighted in bold.

5

Fig. 2 shows the effect of peptide of Example SEQ ID NO. 2 on amyloid cytotoxicity. Soluble amylin (100 μ M), soluble amylin (100 μ M) + peptide of SEQ ID NO. 2 (800 μ M), and peptide of SEQ ID NO. 2 (800 μ M) were pre-incubated at 37°C in Tris buffer pH 7.4 for 24h then diluted in 50 μ l of culture medium before addition to
10 Rin-m5F cells, to yield final concentrations of 2 μ M (amylin) and 16 μ M (peptide of SEQ ID NO. 2). Data are expressed as average MTT reduction \pm SD (n=5) relative to cells treated with medium alone, which was made to equal 100%.

Fig. 3 shows the linear relationship between IC_{50} of peptide of SEQ ID NO. 2 in
15 preventing cytotoxicity in vitro and amylin concentration. Aliquots of 2.5, 10, 50 and 100 μ M of amylin were incubated during 24h at 37°C with different concentrations of peptide of SEQ ID NO. 2. Thereafter, the samples were diluted in cell culture medium and cytotoxicity assay performed as described in Example 2.

20 **Abbreviations**

h (hour), μ l (microliters), μ M (micromolar), mg (milligrammes), min (minutes), ml (milliliters), mM (millimolar), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), nm (nanometers), PBS (Phosphate Buffered Sulfate), pM (picomolar), RT (room temperature), SDS (Sodium Dodecyl Sulfate), TFA (trifluoro
25 acetic acid).

EXAMPLES

The invention will be illustrated by means of the following examples which are not to be construed as limiting the scope of the invention.

30 The following examples illustrate preferred compounds according to Formula I, and methods for determining their biological activities.

Synthetic human amylin (1-37) (TFA salt) and A β ₁₋₄₂ were synthesized in solid phase at W.M. KECK FOUNDATION, YALE UNIVERSITY. Amylin was not chemically reduced to make sure that the cystein bridge between amino acids 2 and 7 was formed. Control peptides, and the prion protein fragment 106-126 were purchased from
5 NEOSYSTEM (Strasbourg). In order to increase the solubility of amylin (1-37), HCl salt of amylin was made by dissolving 1 mg of amylin in 1 ml of 2 mM HCl solution, sonicated for 1 min at RT in an ultrasonic water bath, then lyophilized in aliquots of 0.2 mg and kept dry at 4°C until use.

10 **Example 1: Synthesis of compounds of the invention**

Inhibitor peptides were synthesized in solid phase by NEOSYSTEM. Peptides were purified by HPLC and purity (> 95%) evaluated by peptide sequencing and laser desorption mass spectrometry. Stock solution of the peptides were prepared in water/0.1% trifluoroacetic acid and stored lyophilized in aliquots at -70°C.
15 Concentration of the stock solution was estimated by amino acid analysis.

The chemical derivatization reactions were done during the synthesis by NEOSYSTEM using standard procedures.

The molecular weights measured by mass spectrometry are listed in Table I below:

Table I:

20

SEQ ID N°.	MW (g/mol)
2	619
3	660
4	734
10	720
11	705
12	749
13	699
14	790
15	776
16	733

SEQ ID N°.	MW (g/mol)
17	618

Example 2: Biological assays***In vitro* peptide solubility assay:**

- 5 Solubility of peptides of the invention was obtained using a qualitative assay where the peptide was dissolved in Tris buffer, pH 7.4 at 1, 5, or 10 mg/ml, vortexed briefly, and then centrifuged at 16,000 g for 30 min. The presence of a pellet in the bottom of the tube indicates that the peptide is not soluble at the respective concentration.

The data in Table II below indicate that peptides of the invention are highly soluble:

10

Table II

SEQ ID N°.	Solubility
10	<200µg/ml
11	<1mg/ml
12	2mg/ml
4	>10mg/ml
3	>10mg/ml
2	>10mg/ml

***In vitro* assays of activity:**

The activity of compounds of the invention in inhibiting the formation of aggregated amylin fibrils can be tested by absorbance changes.

15

Amyloid formation was quantitatively evaluated by measuring the amount of bound Congo red (Cb) to the fibrils using the formula below as reported (*Klunk et al., 1999*):

$$\text{Cb } (\mu\text{M}) = (A_{541}/47,800) - (A_{403}/68,300) - (A_{403}/86,200)$$

wherein A_{541} and A_{403} are the absorbances respectively at 541 and 403 nm.

20

Aliquots of amylin at a concentration of 0.4 mg/ml prepared in 50 mM Tris-HCl, pH 7.4 were incubated at 37°C for 48h in the absence or presence of 1 mg/ml of the peptide of the invention. At the end of the incubation time, 125 µl of 15 µM Congo red in PBS was

added to 25 µl of sample, mixed and incubated for 10 min at RT. The absorbance of the resulting solutions was then measured at 403 and 541 nm. The amount of Congo red was calculated using the above formula. The amount of Congo red bound to amylin fibrils in the absence of the inhibitor was set to 100%.

5

The data presented in Table III below indicate that peptides of the invention inhibit the formation of amylin aggregates:

Table III

SEQ ID N°.	% Inhibition of Amylin fibrils
5 (Amylin)	0
12	20
13	16
14	8
15	10
16	24
4	39
3	47
2	43

10

Cellular assays of activity:

The mechanism proposed for the implication of amylin misfolding and aggregation in the pathogenesis of Type II diabetes is by inducing islet β-cell death and thus pancreas dysfunction. The ability of compounds of the invention to prevent or reduce the formation of cytotoxic amylin fibrils (*Lorenzo et al., 1994*) was evaluated by measuring the inhibition of amylin induced cytotoxicity in pancreatic β-cells.

Toxicity was measured by comparing the effects of amylin or amylin combined with peptides of the invention, on the reduction of the redox active dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by PC12 cells or Rin-

20

m5F cells (ATCC), a pancreatic beta cell line. Cells were grown in RPMI-1640 media containing 10% foetal bovine serum, and plated onto 96-well plates.

Amylin at 0.2 mg/ml in 50 mM Tris-HCl pH 7.4, in the absence or presence of 1 mg/ml of peptide of the invention, was incubated at 37°C for 48 h, then diluted in 50 µl of culture media at the appropriate peptide concentration before addition to the Rin-m5F cells. After overnight incubation, 10 µl of 2.5 mg/ml MTT was added to each well and the incubation continued for a further 3 h. Cells were then solubilized in 200 µl of 20% (w/v) SDS in 50% (v/v) *N,N*-dimethylformamide, 25 mM HCl, 2% (v/v) glacial acetic acid, pH 4.7, by overnight incubation at 37 °C. Levels of reduced MTT were determined by measuring the difference in absorbance at 595 and 650 nm using a microplate reader.

Cell viability is measured in presence of amylin alone and for a mixture of amylin and peptides of the invention. Cell viability in presence of a mixture of amylin and compound of SEQ ID NO. 2 is presented in Figure 2 in comparison with amylin alone.

The percentage of inhibition of cellular toxicity is calculated for the mixtures in comparison to cellular toxicity induced by amylin alone. Percentages of inhibition of cellular toxicity are presented in Table IV below for compounds of the invention:

Table IV

SEQ ID N°.	% Inhibition Amylin fibrils cytotoxicity
5 (Amylin)	0
17	19
4	33
3	43
2	40

Since the concentration at which the inhibitory effect of peptides of the invention is observed depends on the concentration of amylin used, the concentration of amylin was varied and the inhibitory concentration at 50% of the effect (IC_{50}) of compound of SEQ ID NO. 2 was calculated. For this, aliquots of 2.5, 10, 50 and 100 µM of amylin were incubated during 24h at 37°C with different concentrations of peptide SEQ ID NO. 2.

Thereafter, the samples were diluted in cell culture medium and cytotoxicity assay performed.

As shown in Figure 3, the IC_{50} for peptide of SEQ ID NO. 2 has a linear relationship with amylin concentration. IC_{50} were found to be 1.5-2.7-fold higher than amylin concentrations used in the study. Considering that amylin concentration in blood has been shown to be 2-3 pM, we estimate that the peptide concentration in plasma in which a 50% of activity would be reached in vivo is 4-6 pM.

Selectivity of the effect of peptides of the invention was assayed in the same assay as presented above.

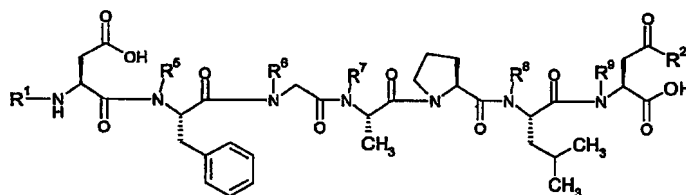
Selectivity of peptides of the invention towards amylin was tested by measuring the ability of peptides of the invention to inhibit cellular toxicity induced by prion protein fragment (PrP₁₀₆₋₁₂₆) by A β ₁₋₄₂ peptide. Peptides of the invention were not able to inhibit either cellular toxicity of prion, nor A β fibrils.

References

- Anguino et al. 2002, *Biochemistry*, 41, 11338-11343;
- Carrell et al. 1997, *The Lancet*, 350, 134-8;
- 5 Cleland et al., *Curr. Opin. Biotechnol.*, 12: 212-9, 2001;
- Cooper GJ et al. 1987, *Proc. Natl. Acad. Sci. U.S.A.*, 84:8628-32;
- Dobson 1999, *Trends Biochem. Sci.* 24, 329-32;
- Fezoui et al. 1999, *Int. J. Exp. Clin. Invest.* 7, 166-178;
- Gennaro et al. 2000, of *Remington's Pharmaceutical Sciences*, Part 8, 20th Edition,
- 10 Mack Publishing Company, Easton, Pennsylvania;
- Glennier et al. 1988, *Biochem. Biophys. Res. Commun* 155, 608;
- Höppener JWM et al. 2000, *N. Engl. J. Med.*, 343, 411-419;
- Karsa et al. 1993, (Ed), *Encapsulation and Controlled Release*; Stephenson (Editor); Springer Verlag;
- 15 Klunk et al. 1999, *Methods Enzymol.*, 309:285-305;
- Lorenzo et al. 1994, *Nature* 368: 756-760;
- Luo et al. 2001, *Exp. Opin. Ther. Patents*, 11: 1395-1410;
- Moriarty et al. 1999, *Biochemistry*, 38, 1811-1818;
- Rink et al. 1993, *Trends in Pharmacol. Sci.* 14, 113-118;
- 20 Soto et al. 2000, *FEBS Lett.*, 498, 204-7;
- Westermarck et al. 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87:5036-40;
- Yacobi et al. 1998, *Oral Sustained Release Formulations: Design and Evaluation*, Bva Halperin-Walega (Editor), 1st Ed. edition; Pergamon Press;
- WO 96/39834, New York University;
- 25 WO 01/34631, Axonyx Inc;
- EP 885904 Fraunhofer Ges. Forschung;
- US 2002/0119926;
- EP 1162207 Amylin Pharmaceuticals;
- US 6,610,824 Amylin Pharmaceuticals.

Claims

1. A peptide having an amino acid sequence of Formula I (SEQ ID NO: 1):
 $X_1FGAPX_2X_3$ in which
 X_1 , is selected from Aspartic acid and a derivative thereof selected from acylated and alkylated Aspartic acid;
 X_2 is Leucine or when X_3 is absent, X_2 is selected from Leucine and amidated Leucine;
 X_3 is absent or selected from Aspartic acid and amidated Aspartic acid;
 as well as salt and any derivative or analogue thereof.
2. A peptide according to claim 1 wherein X_1 and X_3 are Aspartic acid.
3. A peptide according to claims 1 or 2 wherein X_3 is absent.
4. A peptide according to any of the preceding claims of the Formula II below:



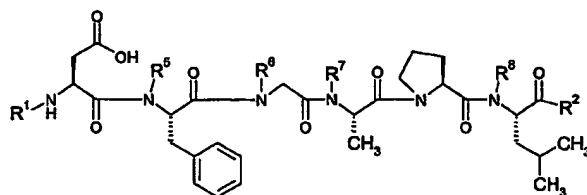
Formula II

wherein R^1 is selected from H, C_2-C_6 acyl and C_1-C_6 alkyl; R^2 is selected from OH and NR^3R^4 , wherein R^3 and R^4 are independently selected from H and C_1-C_6 alkyl and R^5, R^6, R^7, R^8 and R^9 are independently selected from H and C_1-C_6 alkyl.

5. A peptide according to claim 4 wherein R^1 is selected from H and C_2-C_6 acyl and R^2 is selected from OH and NH_2 .

6. A peptide according to claim 4 wherein R^1 is H and R^2 is OH.

7. A peptide according to claim 1 of the Formula III below:



Formula III

wherein R^1 is selected from H, C_2-C_6 acyl and C_1-C_6 alkyl; R^2 is selected from OH and NR^3R^4 , wherein R^3 and R^4 are independently selected from H and C_1-C_6 alkyl and R^5, R^6, R^7 and R^8 are independently selected from H and C_1-C_6 alkyl.

8. A peptide according to claim 7 wherein R^1 is selected from H and C_2-C_6 acyl and R^2 is selected from OH and NH_2 .

9. A peptide according to claims 7 or 8 wherein R^1 is acetyl and R^2 is NH_2 .

10. A peptide according to any of the preceding claims wherein R^5, R^6, R^7 and R^8 are H.

11. A peptide according to any preceding claims selected from the following group: SEQ ID NO. 2, SEQ ID NO. 3 and SEQ ID NO. 4.

12. A compound according to any of the preceding claims for use as a medicament.

13. Use of a peptide according to claims 1 to 11 for the preparation of a medicament for the treatment or prevention of an amyloidosis disorder related to IAPP.

14. Use according to claim 13 wherein the amyloidosis disorder is a diabetic disorder.

15. Use according to claim 14 wherein the diabetic condition is Type II diabetes.

5

16. Use according to claim 14 wherein the diabetic condition is post-transplantation Type I diabetes.

10

17. A pharmaceutical composition comprising a compound according to claims 1 to 11 as active ingredient and a pharmaceutically acceptable excipient or carrier.

18. A method of treating or preventing diabetic disorders by administering an effective amount of any of the peptides or compounds of claims 1 to 11 to a subject in the need thereof.

15

19. A method according to claim 18, in which the subject is human.

Figure 1

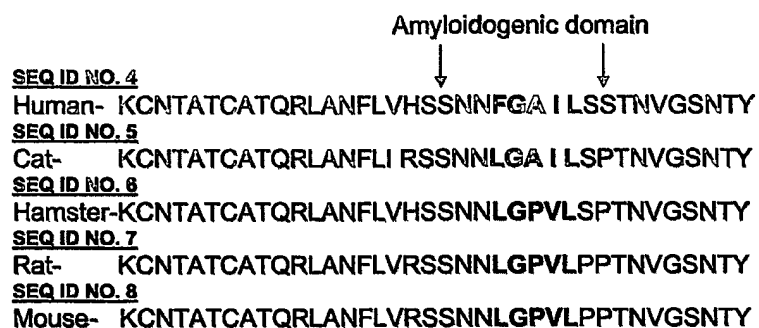


Figure 2

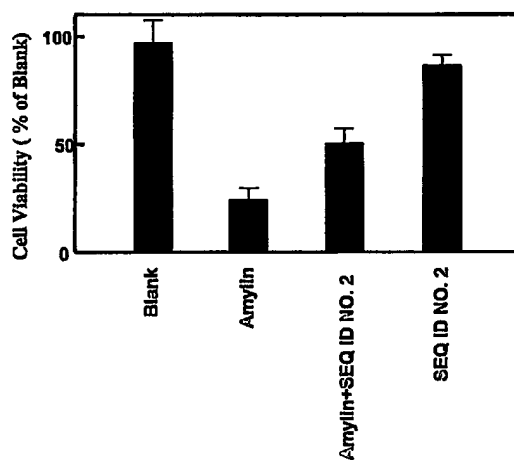
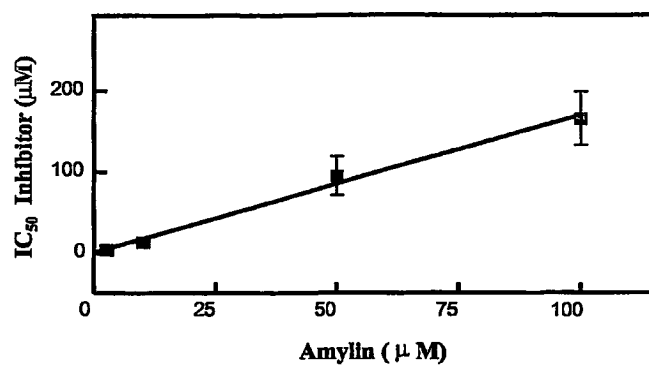


Figure 3



SEQUENCE LISTING

<110> APPLIED RESEARCH SYSTEMS ARS HOLDING N.V.

<120> Amylin aggregation inhibitors and use thereof.

<130> WO/817

<160> 17

<170> PatentIn version 3.1

<210> 1

<211> 7

<212> PRT

<213> synthetic construct

<220>

<221> MISC_FEATURE

<222> (1)..(1)

<223> Xaa, if present, can be Aspartic acid or derivative thereof selected from acylated and alkylated Aspartic Acid;

<220>

<221> MISC_FEATURE

<222> (6)..(6)

<223> Xaa is Leucine or when Xaa in position 7 is absent, Xaa is selected from Leucine and amidated Leucine

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa can be absent or is selected from Aspartic Acid and amidated Aspartic Acid

<400> 1

Xaa Phe Gly Ala Pro Xaa Xaa
1 5

<210> 2

<211> 6

<212> PRT

<213> synthetic construct

<400> 2

Asp Phe Gly Ala Pro Leu
1 5

<210> 3

<211> 6

<212> PRT

<213> synthetic construct

<220>

<221> MISC_FEATURE
<222> (1)..(1)
<223> Xaa is acetylated aspartic acid

<220>
<221> MISC_FEATURE
<222> (6)..(6)
<223> Xaa is amidated Leucine

<400> 3

Xaa Phe Gly Ala Pro Xaa
1 5

<210> 4
<211> 7
<212> PRT
<213> synthetic construct

<400> 4

Asp Phe Gly Ala Pro Leu Asp
1 5

<210> 5
<211> 37
<212> PRT
<213> human

<400> 5

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
1 5 10 15

Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr Asn Val
20 25 30

Gly Ser Asn Thr Tyr
35

<210> 6
<211> 37
<212> PRT
<213> Cat

<400> 6

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
1 5 10 15

Ile Arg Ser Ser Asn Asn Leu Gly Ala Ile Leu Ser Pro Thr Asn Val
20 25 30

Gly Ser Asn Thr Tyr
35

<210> 7
<211> 37
<212> PRT
<213> Hamster

<400> 7

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
1 5 10 15

Val His Ser Ser Asn Asn Leu Gly Pro Val Leu Ser Pro Thr Asn Val
20 25 30

Gly Ser Asn Thr Tyr
35

<210> 8
<211> 37
<212> PRT
<213> Rat

<400> 8

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
1 5 10 15

Val Arg Ser Ser Asn Asn Leu Gly Pro Val Leu Pro Pro Thr Asn Val
20 25 30

Gly Ser Asn Thr Tyr
35

<210> 9
<211> 37
<212> PRT
<213> Mouse

<400> 9

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
1 5 10 15

Val Arg Ser Ser Asn Asn Leu Gly Pro Val Leu Pro Pro Thr Asn Val
20 25 30

Gly Ser Asn Thr Tyr
35

<210> 10
<211> 7
<212> PRT
<213> human

<400> 10

Asn Phe Gly Ala Ile Leu Ser
1 5

<210> 11
<211> 7
<212> PRT
<213> synthetic constuct

<400> 11

Asn Phe Gly Ala Pro Leu Ser
1 5

<210> 12
<211> 7
<212> PRT
<213> synthetic construct

<400> 12

Asp Phe Gly Ala Ile Leu Asp
1 5

<210> 13
<211> 7
<212> PRT
<213> synthetic construt

<400> 13

Asp Pro Gly Ala Ile Leu Asp
1 5

<210> 14
<211> 7
<212> PRT
<213> synthetic construct

<400> 14

Asp Phe Pro Ala Ile Leu Asp
1 5

<210> 15
<211> 7
<212> PRT
<213> synthetic construct

<400> 15

Asp Phe Gly Pro Ile Leu Asp
1 5

<210> 16

<211> 7

<212> PRT

<213> synthetic construct

<400> 16

Asp Phe Gly Ala Ile Pro Asp
1 5

<210> 17

<211> 6

<212> PRT

<213> synthetic construct

<400> 17

Phe Gly Ala Pro Leu Asp
1 5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/050320

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/575 C07K7/06 A61K38/22 A61P5/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, MEDLINE, WPI Data, PAJ, CHEM ABS Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 885 904 A (FRAUNHOFER GES FORSCHUNG) 23 December 1998 (1998-12-23) abstract page 3, paragraph 2	1-19
Y	US 2002/119926 A1 (FRASER PAUL) 29 August 2002 (2002-08-29) abstract; claims; figure 2	1-19
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

6 August 2004

Date of mailing of the international search report

20/08/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Cervigni, S

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2004/050320

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MORIARTY D F ET AL: "Effects of sequential proline substitutions on amyloid formation by human amylin20-29." BIOCHEMISTRY. UNITED STATES 9 FEB 1999, vol. 38, no. 6, 9 February 1999 (1999-02-09), pages 1811-1818, XP002249402 ISSN: 0006-2960 cited in the application abstract page 1817, column 1	1-19
A	WESTERMARK P ET AL: "ISLET AMYLOID POLYPEPTIDE: PINPOINTING AMINO ACID RESIDUES LINKED TO AMYLOID FIBRIL FORMATION" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 87, no. 13, 1 July 1990 (1990-07-01), pages 5036-5040, XP000137094 ISSN: 0027-8424	
Y	WO 96/39834 A (UNIV NEW YORK) 19 December 1996 (1996-12-19) page 25	1-19
Y	SOTO C ET AL: "BETA-SHEET BREAKER PEPTIDES INHIBIT FIBRILLOGENESIS IN A RAT BRAIN MODEL OF AMYLOIDOSIS: IMPLICATIONS FOR ALZHEIMER'S THERAPY" NATURE MEDICINE, NATURE PUBLISHING, CO, US, vol. 4, no. 7, 1998, pages 822-826, XP000914747 ISSN: 1078-8956 page 822, column 1	1-19
A	EP 1 162 207 A (AMYLIN PHARMACEUTICALS INC) 12 December 2001 (2001-12-12)	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2004/050320

Box II Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 18-19
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 18-19 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP2004/050320

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 0885904	A	23-12-1998	DE 19725619 A1	24-12-1998
			AT 262540 T	15-04-2004
			CA 2240005 A1	17-12-1998
			DE 59811036 D1	29-04-2004
			DK 885904 T3	05-07-2004
			EP 0885904 A1	23-12-1998
			JP 11071393 A	16-03-1999
			US 2001007015 A1	05-07-2001
US 2002119926	A1	29-08-2002	AU 9355701 A	02-04-2002
			WO 0224727 A2	28-03-2002
			CA 2422911 A1	28-03-2002
			EP 1325028 A2	09-07-2003
WO 9639834	A	19-12-1996	US 5948763 A	07-09-1999
			AU 715662 B2	10-02-2000
			AU 6112996 A	30-12-1996
			CA 2222690 A1	19-12-1996
			EP 0843516 A1	27-05-1998
			JP 2001519753 T	23-10-2001
			WO 9639834 A1	19-12-1996
			US 2003087407 A1	08-05-2003
			US 6462171 B1	08-10-2002
EP 1162207	A	12-12-2001	EP 1162207 A1	12-12-2001
			AT 205854 T	15-10-2001
			AU 714439 B2	06-01-2000
			AU 1245697 A	27-03-1997
			AU 673147 B2	31-10-1996
			AU 3075392 A	15-06-1993
			CA 2100745 A1	20-05-1993
			CZ 9301689 A3	13-04-1994
			DE 69232064 D1	25-10-2001
			DE 69232064 T2	28-03-2002
			DK 567626 T3	17-12-2001
			EP 0567626 A1	03-11-1993
			ES 2161697 T3	16-12-2001
			FI 933252 A	23-08-1993
			GR 3036794 T3	31-01-2002
			HU 64975 A2	28-03-1994
			JP 11152299 A	08-06-1999
			JP 2902115 B2	07-06-1999
			JP 6504794 T	02-06-1994
			JP 2003238594 A	27-08-2003
			NO 932603 A	17-09-1993
			RU 2130463 C1	20-05-1999
			SK 88793 A3	07-12-1994
			WO 9310146 A1	27-05-1993
			US 5686411 A	11-11-1997
			US 2002187923 A1	12-12-2002
			US 2004038900 A1	26-02-2004
			US 5998367 A	07-12-1999

THIS PAGE BLANK (USPTO)